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Influence of Growth Temperature on Injury and Death of *Listeria monocytogenes* Scott A During a Mild Heat Treatment

ABSTRACT

The growth temperature of *Listeria monocytogenes* has a profound effect on injury and death of washed cells that are suspended in phosphate buffer and exposed to 52°C for 1 h. The temperature of 52°C had low lethality for cells grown at 37 or 42°C, but there was a 10^3-10^4-fold increase in killing for cells grown at 28, 19, 10, or 5°C. There was little injury with exposure to 52°C of cells grown at 5, 10, or 19°C, but injury increased as the temperature of growth increased. When cells were grown anaerobically, lethality induced at 52°C increased as the growth temperature decreased, but there was more injury under anaerobic conditions than for aerobically grown cells. The results indicate that *L. monocytogenes* cells growing at low temperatures are more susceptible to heat induced death.

While the optimum growth temperature for *Listeria* lies between 30-37°C, it can grow between 1 and 45°C (*17*). Such broad temperature ranges indicate that for foods containing listeriae, storage at temperatures above freezing will result in cell multiplication if growth requirements are met. Hansen and Riemann (*11*) reviewed the literature on the effect of growth temperature on heat resistance and concluded that bacterial and yeast heat resistance decreased as the growth temperature was lowered. This fact suggests that microorganisms growing in foods at low temperatures may be killed by lower heating temperatures. When Elliker and Frazier (9) grew *Escherichia coli* at temperatures ranging from 28 to 40°C, they found that fewer *E. coli* survived when heated at 53°C for 30 min if they were grown at the lower temperatures. The D_{55} value for *Salmonella senftenberg* 775W increased as the growth temperature increased from 15 to 44°C (*16*) and similarly, Dega et al. (*6*) showed that salmonellae grown in concentrated milk at 43°C tolerated heating better than those grown at 22°C. Beuchat and Worthington (*3*) found that the D_{47} value increased as the growth temperature for *Vibrio parahaemolyticus* increased from 21 to 37°C. Gram-positive microorganisms behave similarly to gram-negative cells; *Streptococcus faecalis* grown at 45°C were more heat resistant than when grown at 27°C (*22*). Donnelly and Briggs (8 demonstrated that several strains of *L. monocytogenes* grown in milk at temperatures ranging from 4 to 37°C had approximately the same heat resistance regardless of growth temperature, i.e., the $D_{62.7}$ was 1.0 min or less, but Knabel et al. (*13*) indicated that growth of *L. monocytogenes* at temperatures above 37°C (39 to 43°C) gave cells that survived 62.8°C heating better than 37°C grown cells. The cited observations indicate that growing certain microorganisms at lower temperatures results in a population of cells that has decreased heat resistance. However, the results for *L. monoctyogenes* are conflicting and do not not consider the impact that growth temperature could have on sublethal stress caused by thermal injury. The objective of the current study was to determine the effect of growth temperature (5-42°C) on both death and injury of *L. monocytogenes* exposed to a mild heating at 52°C for 1 h.

METHODS AND MATERIALS

Microorganism

Listeria monocytogenes, Scott A strain, was used as a representative strain of the species and was maintained in brain heart infusion broth (BHI; Difco, Detroit, MI) stored at 5°C. Fifty ml of BHI containing additional glucose (BHIG; final glucose concentration, 0.5% w/v) was inoculated with *L. monocytogenes* and incubated on a rotary shaker (150 rpm) at 28°C for 18-20 h. The 28°C grown cells were inoculated into 50 ml BHIG contained in 500-ml flasks and incubated shaken at temperatures ranging from 42 to 5°C. Cells, at the maximum stationary stage (approximately 2.5 x 10^9/ml), were harvested by centrifugation, washed twice with sterile distilled water, and resuspended in 5 ml sterile distilled water. In some experiments, *L. monocytogenes* were grown at different temperatures under N_2 (< 85 ppm 0_2). Trypsinizing flasks (250-ml) with side arms containing sleeve-type rubber stoppers which could be punctured with a syringe needle and containing 25 ml BHI were inoculated with 28°C grown cells and aseptically gassed with nitrogen for 15 min. The loose screw cap (to allow venting) was replaced with a sterile rubber bung at the end of the gassing period. The anaerobic flasks were incubated at various temperatures with shaking. Cells from 100 ml of anaerobic culture, at maximum stationary stage, were washed by centrifugation as described above and resuspended in 5 ml in sterile distilled water.

Injury procedure

Heat treatments were performed using screw-cap 160-ml dilution bottles containing 20 ml sterile potassium phosphate buffer (pH 7.2; 0.1 M). The bottles were equilibrated to 52 ± 0.25°C utilizing a circulating constant temperature water bath; the level of water in the water bath was maintained 1.5 in. above the level of buffer in the bottles. A mercury thermometer was inserted into an uninoculated bottle containing buffer and was checked periodically during the experimental run to ascertain that the temperature was maintained. One ml of washed *L. monocytogenes* was then added to give 2-4 x 10^9 CFU/ml buffer. The buffer-cell suspension was agitated (approximately 150 agitations/min) on a Burrell Wrist-Action Shaker (Burrell, Pittsburgh, PA). At zero time and at 1 h, 0.1 ml of culture was removed from the bottles and added to 9.9 ml sterile 0.1% (w/v) Bacto peptone water tubes and successive dilutions prepared. Using a spiral plater (Spiral Systems Instruments, Inc., Bethesda, MD), appropriate dilutions were plated onto tryptose phosphate broth + 2% agar (w/v) + 1% (w/v) Na pyruvate (TPBAP) and TPBA + 5% (w/v) added NaCl (TPBAS). Plates were incubated at 37°C and counted after 3 d.

Death and injury were monitored by the use of a differential plating technique consisting of TPBAP and TPBAS. Both noninjured cells and injured-repaired cells following repair form colonies on TPBAP, whereas only noninjured cells form colonies on TPBAS; repair of injured cells does not take place in the presence of sodium chloride (*18*).

Death was defined as:

$TPBAP_{0\text{-}h}$ minus $TPBAP_{1\text{-}h}$

and injury was defined as:

($TPBAS_{0\text{-}h}$ minus $TPBAS_{1\text{-}h}$) minus ($TPBAP_{0\text{-}h}$ minus $TPBAP_{1\text{-}h}$).

Statistics

Data were analyzed by one-way analysis of variance using the Ecstatic (Someware in Vermont, Montpelier, VT) and Number Crunching Statistical System (J.L. Hintze, Kaysville, UT) statistical software programs.

RESULTS

The injury temperature used, 52°C for 1 h, had low lethality for washed cells of *L. monocytogenes* grown at 37°C or 42°C under aerobic conditions but was lethal for cells grown at 28°C or lower (Table 1; Fig. 1). The decrease in population (death) for cells grown at 28°C or lower was 10^3-10^4 times more than that for 37°C grown cells. However, more injury was seen when the cells were grown at higher temperatures than at lower temperatures (Table 1; Fig. 1). Similarly, *L. monocytogenes* grown under anaerobic conditions showed increasing lethality as the growth temperature was lowered (Table 2; Fig. 1); however, injury appeared to be less affected by growth temperature under anaerobic conditions.

When aerobically and anaerobically grown *L. monocytogenes* were compared, more cell destruction occurred at 52°C for cells grown at 37°C anaerobically as compared to cells grown aerobically at 37°C (Fig. 1), while cell destruction for 28 and 19°C anaerobically grown cells was less than that for aerobically grown cells. Aerobically, injury decreased as the growth temperature decreased but growth temperature appeared to have less effect on injury anaerobically (Fig. 1). However, the overall pattern that lower growth temperatures produced cells more likely to be killed by a 52°C heat treatment was observed with both aerobically and anaerobically cultured cells.

DISCUSSION

Thermal injury or death of microbial cells may result from physical or biochemical alterations that inhibit cell division, substrate uptake, metabolism, or bioenergetic mechanisms (2). Growth temperature also appears to be a factor in determining the lethality when the cells are subjected to heat treatment. Previous workers have shown that microorganisms grown at higher temperatures survive heating better than when grown at lower temperatures (*3,6,9,13,16,22*).

The data presented here show that *L. monocytogenes* Scott A behaves similarly to other microorganisms; there is decreased heat resistance as the growth temperature is decreased under both aerobic and anaerobic conditions. The mechanism as to why growth at lower temperature leads to cells with decreased heat resistance is unknown. However, many workers have shown that microorganisms increase their unsaturated fatty acid content as the growth temperature is lowered. For example, Marr and Ingraham (*15*), Haest et al. (*10*), and Katsui et al. (*12*) found that as the growth temperature for *E. coli* decreased, the proportion of unsaturated fatty acids increased. Similar results were obtained for *L. monocytogenes* (*19*), *V. parahaemolyticus* (*3*), some strains of psychrophilic *Vibrio* (*4,7*), *Yersinia enterocolitica* (*1,21*), *S. faecalis* (*23*), *Pseudomonas fluorescens* (*5*) and *Bacillus stearothermophilus* (*14*). Thus, the general microbial response to decreased growth temperatures appears to be an increase in the level of unsaturated fatty acids in the phospholipids present in the cytoplasmic membrane, resulting in a more fluid, less viscous membrane. Using *V. parahaemolyticus*, Beuchat and Worthington (*3*) demon-

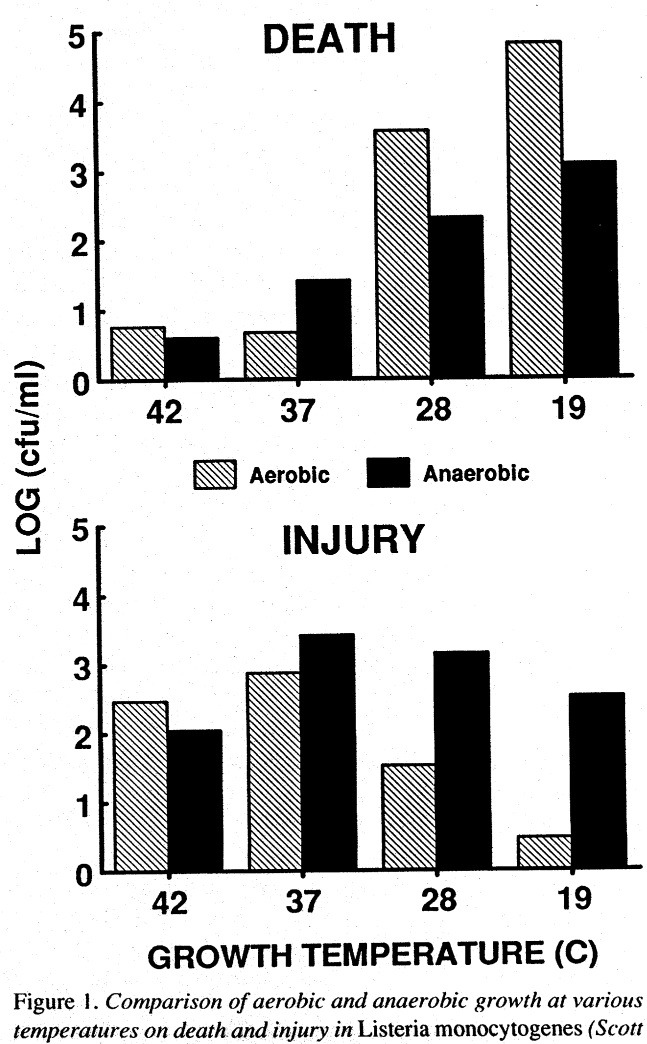

Figure 1. *Comparison of aerobic and anaerobic growth at various temperatures on death and injury in* Listeria monocytogenes *(Scott A) at 52°C in phosphate buffer.*

TABLE 1. *Effect of growth temperature under aerobic conditions on* Listeria monocytogenes *(Scott A) death and injury at 52°C in phosphate buffer.*

		Log_{10} CFU/ml at zero time		Log_{10} CFU/ml at 60 min		Log_{10} CFU/ml	
Growth temp C	n[a]	TPBA+P	TPBA+S	TPBA+P	TPBA+S	death[b]	injury[b]
42.0	11	9.04 (0.19)[c]	8.95 (0.22)	8.28 (0.49)	5.72 (0.66)	0.76 (0.46)[1]	2.47 (0.58)[3]
37.0	35	9.38 (0.18)	9.31 (0.17)	8.72 (0.30)	5.77 (0.64)	0.67 (0.31)[1]	2.88 (0.60)[4]
28.0	16	9.55 (0.15)	9.49 (0.17)	6.06 (0.51)	4.42 (0.73)	3.57 (0.40)[2]	1.58 (0.78)[2]
19.0	16	9.45 (0.20)	9.40 (0.21)	4.60 (0.82)	4.18 (0.74)	4.82 (0.90)[3]	0.47 (0.49)[1]
10.0	11	9.26 (0.18)	9.28 (0.14)	4.18 (0.58)	3.80 (0.56)	5.08 (0.64)[3]	0.40 (0.41)[1]
5.0	10	9.11 (0.09)	9.13 (0.14)	4.47 (0.64)	4.09 (0.68)	4.64 (0.67)[3]	0.40 (0.24)[1]

[a]n = number of replicates.
[b]Means followed by the same superscripted number in columns are not significantly different ($p < 0.05$); Fisher's LSD Comparison.
[c]Numbers in parentheses are standard deviation of means.

TABLE 2. *Effect of growth temperature under aerobic conditions on* Listeria monocytogenes *(Scott A) death and injury at 52°C in phosphate buffer.*

		Log_{10} CFU/ml at zero time		Log_{10} CFU/ml at 60 min		Log_{10} CFU/ml	
Growth temp C	n[a]	TPBA+P	TPBA+S	TPBA+P	TPBA+S	death[b]	injury[b]
42.0	12	9.38 (0.66)[c]	9.33 (0.26)	8.72 (0.44)	6.68 (0.80)	0.60 (0.64)[1]	2.05 (0.51)[1]
37.0	12	9.22 (0.27)	9.05 (0.17)	7.80 (0.33)	4.20 (0.63)	1.42 (0.42)[2]	3.42 (0.63)[2]
28.0	16	9.24 (0.16)	9.03 (0.15)	6.94 (0.53)	3.58 (0.50)	2.31 (0.54)[3]	3.15 (0.72)[2]
19.0	18	9.62 (0.11)	9.54 (0.18)	6.53 (0.73)	3.91 (0.52)	3.09 (0.71)[4]	2.52 (0.93)[1]

For explanation of footnotes a, b, and c, see Table 1.

strated that higher heat resistance correlated with higher growth temperature as well as with decreased unsaturation in the phospholipids. They suggested that a cytoplasmic membrane with decreased unsaturation (brought about by higher growth temperatures) was important in maintaining the resistance of the cells to heat treatment. Yatvin (*25*) suggested that increased fluidity of membrane lipids (contain more unsaturated fatty acids) due to growth at low temperatures is a major factor contributing to the death of cells when exposed to heat.

Yamamori and Yura (*24*) showed that heat resistance was conferred on an *E. coli* strain growing at 30°C when it was preincubated for 30 min at 42°C. Chloramphenicol, an inhibitor of protein synthesis, inhibited the heat resistance effect when added to the cells before temperature shift. They attributed the protective effect against heat to the synthesis of heat shock protein(s). When a *Sulfolobus* species grown at 70°C was incubated at 88°C, they were resistant to a 92°C heat treatment lethal to cells grown only at 70°C (*20*). These authors attributed the increase in thermotolerance to the synthesis of specific proteins at 88°C; however, these proteins did not appear to be typical heat shock proteins (*20*). Recent experiments in our laboratory have demonstrated that shifting *L. monocytogenes* grown at 19°C to 37°C for 2.5-5.0 h increased the heat resistance of the cells, i.e., 19°C grown cells were approximately 100-fold more resistant to heating at 52°C if they were given a 2.5-5.0 h incubation at 37°C. The protective effect was prevented if chloramphenicol was present during the shift-up period (J.L. Smith and B.S. Marmer, manuscript in preparation). It seems likely that the shift from 19°C to 37°C induces the synthesis of a protective protein whose synthesis is inhibited by chloramphenicol.

The mechanism underlying the decreased heat resistance found in cells of *L. monocytogenes* when grown at lower temperatures (≤28°C) remains obscure. Regardless of the mechanism, the data presented here suggest that foods stored at low temperatures which contain growing *L. monocytogenes* can have cells more susceptible to killing by decreased heat processing conditions.

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